

See Colour Plates 30, 31.

See also: II/Chromatography: Thin-Layer (Planar): Densitometry and Image Analysis; Mass Spectrometry; Modes of Development: Conventional; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal; Spray Reagents. III/Flame Ionization Detection: Thin-Layer (Planar) Chromatography. Thin-Layer Chromatography-Vibration Spectroscopy. Appendix 2 / Essential Guides to Method Development in Thin-Layer (Planar) Chromatography.

Further Reading

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Ion Pair Thin-Layer (Planar) Chromatography

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Introduction

The separation of polar, ionizable substances can cause problems for simple partition- or adsorption-based chromatographic systems and this is as true in thin-layer, or planar, chromatography (TLC) as for any of the other liquid chromatographic techniques. Often the problem can be dealt with by the simple expedient of controlling the pH of the solvent used for chromatography, in order to suppress the ionization of the solute and thereby reduce its polarity. However, with very polar compounds this is not always a successful strategy and in such circumstances it may be more practical to employ an ion pair (IP) reagent (often referred to as soap chromatography in the early literature). A further benefit of using such an approach is that a hydrophobic IP reagent may, in addition to masking the ionizable group, usefully modify the overall polarity of the complex and thus cause a dramatic change in chromatographic properties. Whilst IP-TLC has not been as widely employed as IP-high performance liquid chromatography (IP-HPLC: described elsewhere in this encyclopedia), there have been sufficient examples of the technique to demonstrate its utility for both acids and bases.

In addition to simply using IP reagents in order to obtain suitable chromatographic separations for substances that are too polar to chromatograph in any other way, this type of TLC has other uses. For example, faced with a mixture of neutral and ionizable compounds, an IP reagent may enable the ana-

lyst to introduce additional selectivity into the system. This can enable the R_f of more acidic or basic compounds to be altered whilst leaving those of neutral solutes unchanged and thereby effecting a separation. The addition of an IP reagent to a system can also provide a rapid indication of which compounds in a mixture are ionizable. There have also been several examples of the use of chiral IP reagents for the separation of enantiomers.

The scope and practice of IP-TLC are described below.

Practical Aspects of IP-TLC

Suitable Ion Pair Reagents for IP-TLC

A range of IP reagents have been used for TLC and high performance TLC (HPTLC). These include both the conventional IP reagents used in other forms of liquid chromatography, such as heptanesulfonic acid, triethanolamine dodecylbenzene sulfonate and sodium dodecyl sulfate for bases and quaternary ammonium salts including tetramethyl, tetrabutylammonium and cetrimide for acids. Some limited work has been performed using 'bolaform' bisquaternary ammonium compounds where two such groups are separated by an alkyl chain of varying length which acts as a spacer.

In addition to these IP reagents, it is arguable that in cases where strong organic acids such as trifluoroacetic acid have been used to enable good chromatography of bases to be obtained, a form of IP chromatography is being performed.

Chiral IP reagents have been employed in a limited number of cases (see below) in order to effect the resolution of certain basic drugs.

Methods for the Use of IP Reagents

When attempting to perform IP-TLC it is worth noting that there are several methods of using the reagents. The best results depend to no small degree on the nature of the reagent and the stationary phases employed. Thus the reagents can be included as additives to the solvent system, can be applied to the plates via impregnation with a suitable concentration of the reagent in a volatile solvent prior to chromatography, or a combination of inclusion in the mobile phase and impregnation. Thus, in the case of small, polar IP reagents such as the quaternary tetramethylammonium salts, inclusion in the mobile phase is essential if chromatography is being attempted on a bonded-silica stationary phase. This is because the reagent is so polar that it is not retained on the stationary phase but migrates with the solvent front. The reagent is thus rapidly removed from the analytes with which the ion pairing is desired, with the result that there is little effect on chromatography. In contrast, any attempt to use the combination of a long chain IP reagent such as sodium dodecyl sulfate or cetrime as a mobile-phase additive in combination with a bonded phase will invariably be met with disappointing results. This is a direct result of the strong interaction of the nonhydrophilic side chain with the stationary phase, which results in the slow migration of the reagent (if it migrates at all) compared to the solvent. Thus, by the time the advancing solvent front reaches the spots corresponding to the analytes, the concentrations of IP reagent are negligible and no real effect on the subsequent chromatography is seen. For such reagents the prechromatographic impregnation of the stationary phase is essential.

Some of these effects, which illustrate the importance of the correct use of the reagent, are shown in **Figure 1** for a number of dihydroxybenzoic acids with C_2 -bonded HPTLC plates when the quaternary ammonium IP reagent tetrabutylammonium bromide was used. Using the reagent in the mobile phase alone gave unsatisfactory results: there was either little effect or the production of double spots (for ion-paired and nonpaired solute; **Figure 1A**). When the C_2 phase was impregnated with the reagent prior to chromatography, much better results were obtained (**Figure 1B**); maximum effects were seen when the reagent was impregnated into the stationary phase and included in the solvent (**Figure 1C**). Postchromatography, the zones of the plate where the reagent was present were located, due to the lack of a suitable chromophore, using iodine vapour. This provides a simple, rapid and effective method for determining the location of these reagents. This can be important in troubleshooting a separation where the effects obtained are not those anticipated.

The impregnation procedure used can affect the final result and detailed studies have been undertaken to optimize this step. For example, if a paraffin-impregnated silica gel TLC plate is to be used with an IP reagent, there would be an obvious benefit to performing paraffin and IP impregnation with a single solution. However, it has been shown that dipping the plate in paraffin followed by the IP reagent solution results in a much higher quantity of IP reagent being adsorbed than the simultaneous application of paraffin and reagent.

Concentration Effects

In our experience, one of the most important factors in making IP-TLC effective is the concentration of the

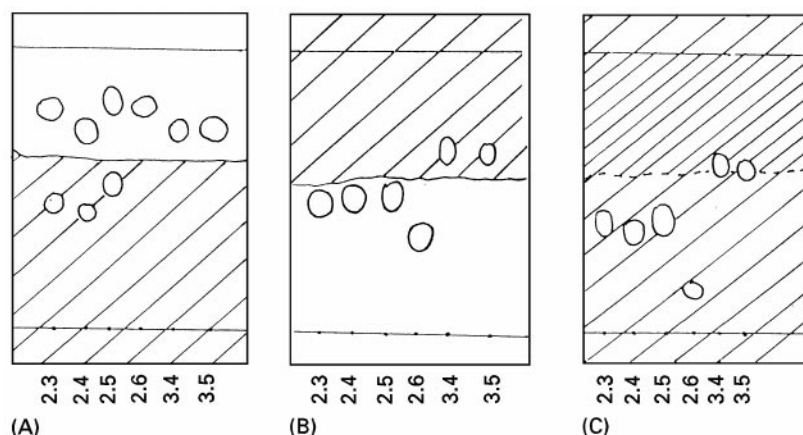


Figure 1 The distribution of IP reagent (tetrabutylammonium bromide) following chromatography as detected using iodine vapour. Shaded areas show the highest concentrations of iodine and thus ion-pair reagent. 2.3, 2.4, 2.5, 2.6, 3.4 and 3.5 are the dihydroxybenzoic acids used as model compounds.

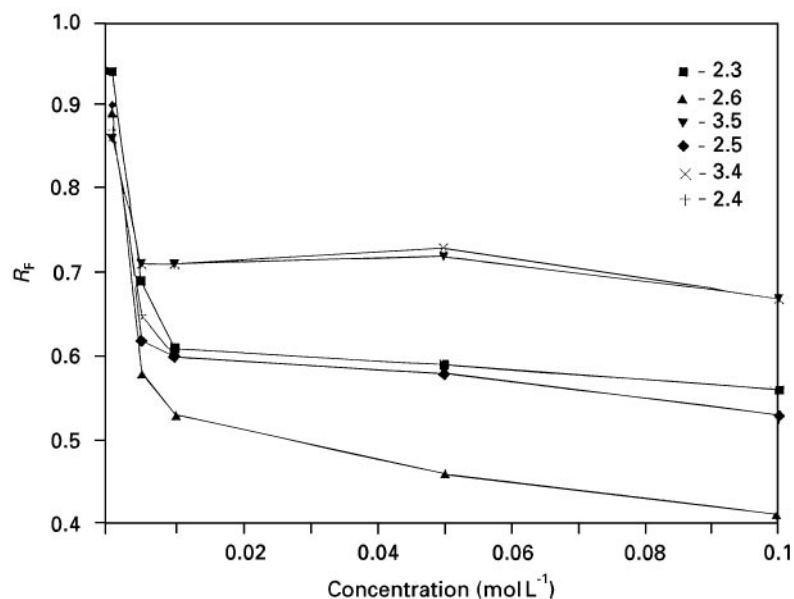


Figure 2 Effect of the concentration of tetrabutylammonium bromide used to impregnate C₂-bonded reversed-phase TLC plates on the R_F values of a range of dihydroxybenzoic acids.

reagent employed. This is shown in Figure 2 for the reagent tetrabutyl ammonium bromide used for a range of dihydroxybenzoic acids on C₂-bonded reversed-phase (RP)-TLC plates. As this figure shows, with methanol-water (1:1 v/v) as mobile phase when no reagent is present the test compounds all have R_F values in the range 0.8–0.9. Prechromatographic impregnation of the phase with a solution of the reagent in dichloromethane–ethanol (85:15 v/v) at 0.001 mol L⁻¹ was virtually without effect. However, increasing the concentration of the impregnating solution to 0.05, 0.01, 0.5 and 0.1 mol L⁻¹ produced significant reductions in R_F that increased with increasing reagent concentration. Above 0.01 mol L⁻¹ the reduction in R_F observed does not dramatically increase with increasing concentration.

The Role of pH

In IP chromatography in HPLC, the careful control of pH is probably the single most important factor in obtaining retention and ensuring reproducible results. Surprisingly, this is not always the case in IP-TLC and pH effects are often of only marginal significance. In general, with IP reagents such as tetrabutylammonium salts, effects of pH are not observed at all over the range 2–10 (Figure 3). For effects over the range 2–4, there is a gradual decline on silica gel and paraffin-coated silica gel plates and a general decline in R_F is observed with increasing pH on C₁₈-bonded phases (Figure 4). Some of these differences in behaviour between IP reagents may be explained in terms of the way in which the reagents interact with the sta-

tionary phases. A working hypothesis that has been advanced is that on silica gel a reagent such as cetrimide is likely to bond ionically to the acidic surface silanols to form a hydrophobic layer in which the hydrophobic alkyl chains of further cetrimide molecules can sit. As long as the silanols on the silica surface remain ionized this would effectively make a relatively stable ion exchange phase (Figure 5). However, as the pH is lowered, such interactions become less favoured and the layer breaks down with a consequent rapid increase in the R_F of polar solutes. IP reagents such as the tetramethyl- or tetrabutylammonium compounds, which have very different physicochemical properties to cetrimide, would seem at first glance to be much less likely to form this type of ordered layer structure, and so the effect of solvent pH on the surface silanols of the stationary phase would be likely to be less important. Whatever the cause, the susceptibility of IP-TLC to pH effects seems to require the presence of a long aliphatic chain as they are only observed with quaternary ammonium trimethyl-*N*-alkyl IP reagents of C₈ or longer.

It may be advantageous under some circumstances to be able to choose between IP systems that are to all intents and purposes insensitive to solvent pH and those with which pH may be used as another parameter with which to manipulate the separation.

Stationary Phases for IP-TLC

A range of stationary phases have been used for IP-TLC including silica gel, paraffin-coated silica gel

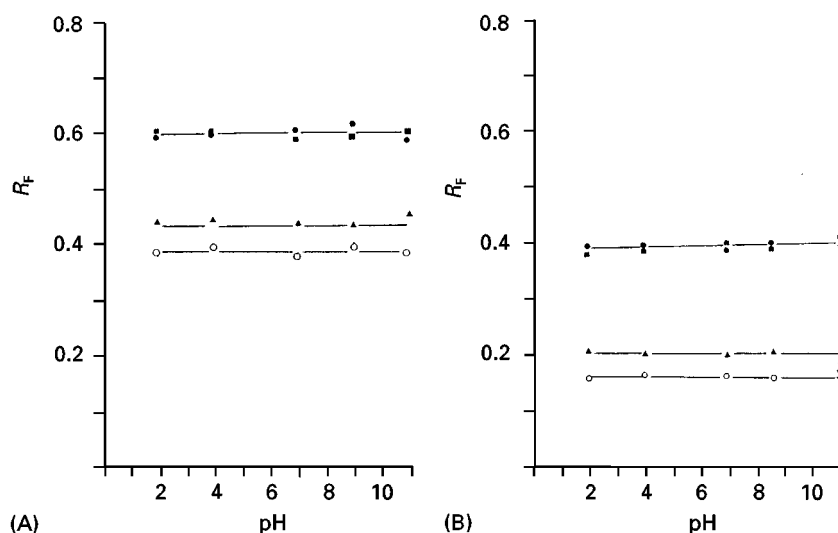


Figure 3 Lack of effect of pH on the R_F values of some dihydroxybenzoic acids on IP-TLC using tetrabutylammonium reagents on (A) paraffin-coated silica gel TLC plates and (B) C_{18} -bonded TLC plates. ■, gentisic acid; ●, 2,5-dihydroxybenzoic acid; ○, 2,6-dihydroxybenzoic acid; ▲, salicylic acid.

and a range from C_2 , C_8 , cyanopropyl, aminopropyl, diphenyl to C_{18} -bonded layers. Chromatography of polar ionic analytes can be obtained with a range of IP reagents on silica gel, paraffin-coated silica gel, alkyl-bonded (C_2 to C_{18}) and diphenyl-bonded layers. However, it is our experience that aminopropyl layers give poor results with IP reagents, and that spot shapes on cyanopropyl layers are poor. It should be noted that these comments are based on limited studies and relatively little work has been done to investigate the use of some of these layers (e.g. cyanopropyl, aminopropyl and diphenyl). It should also be noted that not all reagents can be used with all phases. For example, tetrabutylammonium bromide can be employed with alkyl-bonded stationary phases or paraffin-coated silica gel, but is ineffective on bare silica. In

contrast, cetrимide works well on both bonded stationary phases and silica gel.

Quaternary Ammonium IP Reagents for the TLC of Acids

Reverse-phase IP-TLC In general, the TLC of weakly acidic compounds (e.g. nonsteroidal anti-inflammatory drugs such as ibuprofen or naproxen) can be performed on C_{18} -bonded layers without the need for ion pairing, or indeed for the need for an acidic mobile phase to control the ionization of the analytes. This contrasts with the experience of obtaining satisfactory retention in HPLC for such compounds, but this merely serves to reinforce the fact that the important factors governing retention in RP-TLC and RP-HPLC are not necessarily the same. This may perhaps

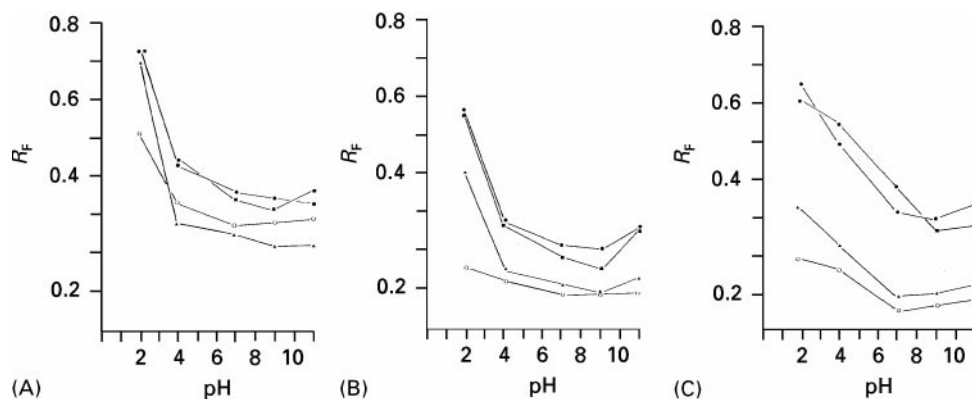


Figure 4 Effect of pH on the R_F values of some dihydroxybenzoic acids on (A) silica gel coated with cetrимide, (B) paraffin-coated silica gel and cetrимide and (C) C_{18} -bonded silica gel and cetrимide.

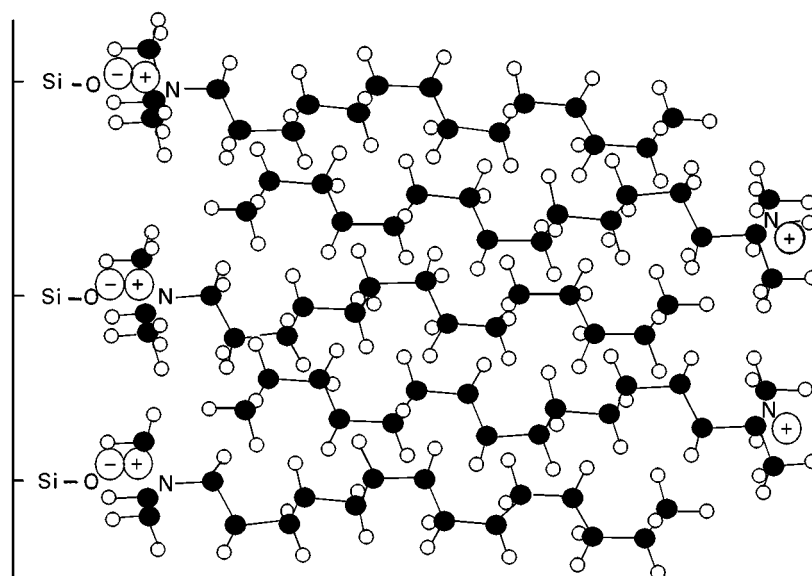


Figure 5 A schematic representation of the possible interaction of long chain IP-reagents with silica gel.

be a result of the acidic nature of the silica surface remaining after the bonding process: this may suppress the ionization of these weak organic acids. This is not to say that IP reagents have no effect on the TLC of such compounds – far from it – and IP reagents can be used to modify R_F for these analytes if required. However, in the case of the RP-TLC of strongly acidic compounds, there is no doubt that the chromatography of such analytes greatly benefits from the use of an IP reagent (and may not be possible without them). Examples of the type of compound where significantly better results are obtained with the employment of an IP reagent (either in terms of ability to modulate R_F or improvements in spot shape) include substances such as mono- and dihydroxylated benzoic acids (e.g. salicylic acid, gentisic acid, etc.). Suitable reagents for the IP-TLC of acidic compounds include tetramethylammonium and tetrabutylammonium salts, cetrimide and the bola-form IP reagents alluded to above.

Acidic IP Reagents for the TLC of Bases

A number of applications of the use of IP-TLC for the chromatography of bases have also been described. That said, it is our experience that the TLC (both normal-phase (NP) and RP) basic compounds can often be performed without recourse to IP systems. Thus, the inclusion of ammonia in NP-TLC systems is often enough to suppress the ionization of weak bases and obtain good chromatography. In RP systems the main problem is usually spot tailing, as the basic analyte interacts with the residual silanols present on

the silica surface. This effect can usually be controlled by using a competing base such as ammonia or triethylamine in the eluent. Alternatively a strong organic acid, such as trifluoroacetic acid (TFA), can be used as a mobile-phase additive (0.1% v/v). It is arguable that this is in fact an ion pairing effect and that the TFA is forming an IP with the basic group on the analyte, thus preventing silanophilic interactions. In HPLC the IP chromatography of bases is usually effected using sulfonic acids such as heptanesulfonic acid and the longer chain dodecylsulfate. Studies have been performed with these reagents on silica gel, silanized silica gel, paraffin-coated silica gel and a range of C_{18} -bonded phases, with the best results usually obtained by coating the reagents on to the plate rather than including them in the mobile phase.

In an early study silanized silica gel was used and the reagents (sodium lauryl ethersulfate or triethanolamine dodecylbenzenesulfonate) were mixed with the stationary phase prior to the preparation of the plates. The amount of reagent employed varied from 0.025% up to 4%, with the maximum effect on the R_F of the test amines seen between 2 and 3%. Later studies showed that a range of reagents, from methyl up to dodecyl sulfonic acids, could be employed with C_{18} -bonded plates and acetone–water (0.1 mol L⁻¹ IP reagent) solvent system for the IP-TLC of analytes such as atropine, codeine, eupaverin and papaverine. Not surprisingly, the authors found that the trend for decreasing R_F of the test solutes was correlated with increasing alkyl chain length for the reagent.

In our own studies the IP-TLC of β -blockers was investigated using silica gel, paraffin-coated silica gel

or C_{18} plates impregnated with sodium dodecyl sulfate. In fact it was possible to obtain good results for these analytes only on the C_{18} -bonded materials. The largest effects on chromatographic behaviour were seen for the dodecyl sulfate reagent.

Overpressure IP-TLC Overpressure TLC (described in detail elsewhere in this Encyclopedia) is a forced flow planar chromatography system whereby the TLC plate is held under pressure beneath an inert and impermeable membrane and the mobile phase is pumped through the layer. This mode of TLC has been used successfully with quaternary ammonium IP reagents using both silica gel and bonded stationary phases and with 10-camphor sulfonic acid on silica gel. When using a reagent such as cetrimide the results were shown to be only really acceptable if the plates were precoated with the reagent, at a concentration of 0.05 mol dm^{-3} . However, if in addition to coating the plates, cetrimide (optimally 0.1 mol dm^{-3}) was also included in the developing solvent, a noticeable improvement in spot shape was seen. Interestingly, and unlike the situation observed for normal IP-TLC, a noticeable dependence of R_F on pH was noted: best results depended upon the stationary phase. So, with bare silica pH 5 gave the best separations whilst on a C_{18} -bonded silica pH 8 was optimal. Later work extended the use of IP-overpressured liquid chromatography (IP-OPLC) to basic compounds with 10-camphor sulfonic acid as the IP reagent, with silica gel as the stationary phase. Interestingly, the investigators noted that this reagent was ineffective when used alone (impregnated into the silica gel by immersion, with or without the reagent also present in the solvent). However, when the plates had previously been impregnated with cetrimide, good results were obtained. This was interpreted as the formation of a double layer of some description. Presumably the cetrimide interacts with the silanols on the surface of the silica gel to form a lipophilic layer on to which the camphor sulfonic acid can be adsorbed. Optimum results are obtained if the plates are pretreated with 0.05 mol L^{-1} solutions of the reagents and both cetrimide and 10-camphor sulfonic acid are present in the eluent at the same concentration. pH effects were also noted in this work, with the best results seen at pH 5.5.

Chiral IP-TLC IP reagents have been used in a limited number of cases in HPLC for the resolution of enantiomers and similar work has also been performed in TLC. Here the aim is to form diastereoisomeric complexes with different chromatographic mobilities rather than mask polar ionizable groups in order to moderate polarity. A number

of IP methods for β -blockers such as propranolol and alprenolol have been reported, based on the use of *N*-benoxycarbonyl-glycyl-L-proline (ZGP) and a diol-bonded HPTLC phase. These plates are firstly pre-washed with dichloromethane and then dipped in the mobile phase to impregnate them with the reagent. The mobile phase consists of a 5 mmol L^{-1} solution of ZGP and 0.4 mmol L^{-1} ethanolamine in dichloromethane. In one method the samples were applied to the plate followed by continuous developments to *c.* 4 cm for 10–20 min. The plates were then dried and the process repeated. Solvent composition for this type of IP-TLC can be critical, and in our hands the inclusion of 2% v/v ethanol to the dichloromethane is essential in order to obtain separation of the enantiomers. With less than this amount of ethanol, no resolution is obtained whilst higher proportions result in increased R_F values for the analytes and a loss of resolution. Sample loading is also an important factor: the best results are obtained with loadings of propranolol of *ca.* $5 \mu\text{g}$ applied as a 1 cm band.

Conclusions

IP-TLC can be used to advantage for the modulation of the chromatography of acidic and basic analytes on a range of stationary phases. The way in which the reagents are used (i.e. in the mobile phase or impregnated on to the plate) depends on the structure of the reagent itself and the nature of the stationary phase. Probably the most important factors controlling the magnitude of the effect obtained when using an IP reagent in planar chromatography are the structure of the reagent (with long chain reagents giving the biggest effects) and the concentration of reagent employed. In contrast to HPLC, pH has relatively little effect in many IP-TLC systems.

See also: II/Chromatography: Liquid: Ion Pair Liquid Chromatography. **Chromatography: Thin-Layer (Planar):** Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal. **III / Acids:** Thin-Layer (Planar) Chromatography. **Bases: Thin-Layer (Planar) Chromatography. Chiral Separations:** Ion-Pair Chromatography; Thin-Layer (Planar) Chromatography. **Impregnation Techniques: Thin-Layer (Planar) Chromatography.**

Further Reading

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Layers

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Although many new tools, such as high performance liquid chromatography (HPLC) and capillary zone electrophoresis, have been developed for the analytical laboratory, none can be used as quickly or inexpensively as planar or thin-layer chromatography (TLC). This tool can be used as a complete analytical system (from initial separation to final quantitation) or as a stepping stone or aid for other analytical methods. It is one of the few analytical tools that requires no sample clean-up, saving time and money. Many samples can be applied and analysed at the same time on a single TLC plate. With the combinatorial chemistries now being used to develop new drugs, renewed interest in this high throughput tool has become apparent.

One distinct advantage, often overlooked, is that when using TLC for any screening or method development, it shows components at the origin that do not move and will over time contaminate other analytical systems, such as an HPLC column. This potential problem would not be apparent if optimizing solely on the HPLC system. Likewise, samples applied after sample preparation would show whether or not sufficient clean-up was actually accomplished with the sample preparation technique used. This can save countless hours of frustration that would develop long after the initial method was thought to be sufficient for the analytical results needed.

Planar chromatography is also useful when optimizing a solid-phase extraction (SPE) method, when

many SPE tubes and extracting solvents will be tried. Up to 20 samples can be spotted on each side of a 20 × 20 cm TLC plate (Figure 1). Each side can be developed in 10–20 min to just 6–8 cm. A quick look under UV light or spray visualization can show the presence or absence of a compound of interest. This is certainly many times faster than doing the 40 samples on an HPLC system, one at time.

Samples from any fast TLC screening which are found to be positive for the component of interest can be confirmed or further quantified with TLC by applying standards and using densitometry or can be transferred to another analytical technique.

This is a brief review of the layers most often used and referenced in planar chromatography. For details on less used layers or more information on those discussed, the reader is referred to the Stahl book listed in the Further Reading section. A few recommended methods or tips are included within each section, where their use will improve performance or the property of a plate or separation.

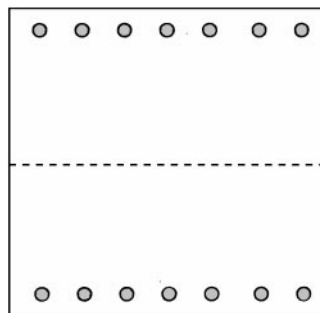


Figure 1 Multi-spotted 20 × 20 cm TLC plate.